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Changes in the Activity of Digestive Enzymes in Response to Chemical Mutagen Diethyl Sulfate in the Silkworm *Bombyx mori* L. (Lepidoptera: Bombycidae)

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ABSTRACT

The silkworm *Bombyx mori* L. is a economic sericigenous insect show a high degree of sensitivity towards chemical mutagens and have drastic physiological and biochemical effects during its larval stages. In the present study a bivoltine race of silkworm NB_4D_2 was treated with chemical Mutagen Diethyl Sulfate (DES). The larvae were used for the treatment with different concentrations of DES orally as well as injected through body wall. In F_1 generation, the larvae of 4th and 5th instar were used to study the activity of prominent digestive enzymes amylase and protease for evaluation of the mutagenic response in haemolymph and midgut tissues. The enzyme activity was found to be higher in treated sets, particularly in 8 mM injection sets. Further, maximum enzyme activity was noticed during middle part of both the instars with a few variations among the treated sets. The data were statistically analysed using One-way ANOVA and discussed.

Key words: *Bombyx mori*, diethyl sulfate (DES), midgut, haemolymph, amylase, protease, NB_4D_2

INTRODUCTION

The *Bombyx mori* L. is an important economic sericigenous insect which feed mainly mulberry leaf and convert leaf protein into silk protein (Babu *et al*., 2009). In silkworm, majority of the characters that contribute to the yield of silk are under the control of polygenic nature (Seshagiri *et al*., 2009; Konate *et al*., 2011). Enzymes play a vital role in the metabolism of dietary food in the body of an organism. Many investigators have carried out the biochemical studies with respect to role of enzymes in various stages of the metamorphosis of insects. The biochemical parameters have been proved to be valuable tools for studying genetic variation in natural populations and have been used as useful indicators in plant and animal breeding programme (Tanksley *et al*., 1982). In silkworms, the digestive enzymes in the midgut breakdown the complex form of nutrients present in the food into simpler forms. These simpler forms are easily absorbed into the body through the semi permeable membrane of alimentary canal.

Amylase are the important enzymes, involved in the metabolism of carbohydrates in silkworms. The two forms of hydrolases, α-amylase and α-glucosidase are key enzymes involved in starch breakdown and absorption, respectively. It is now believed that inhibition of these enzymes involved in the digestion and uptake of carbohydrates can significantly alter (Ashutosh and Jha, 2011; Jeroh *et al*., 2011) the total carbohydrate level in the midgut and hemolymph. The quantitative, qualitative and functional differences between the two amylase types have been

shown by Matsumura (1930). Fundamental localization and the activities of digestive enzymes such as amylase and protease in different tissues have been studied in detail by many authors (Horie *et al*., 1963; Chatterjee *et al*., 1993; Kumari *et al*., 1997). Matsumura (1930) and Kikkawa (1953) have pointed out a possible regulation with two different genes (Ae and Be) for the control of amylase functions in digestive and body fluids of different races of silkworm. Digestive amylase was identified as an excellent marker for silkworm breeding in view of its genetic divergence, its role in better digestibility and close association with survival. Different amylase enzymes were identified by analysis of digestive fluid and haemolymph in different strains of silkworm, *Bombyx mori* (Abraham *et al*., 1992). Proteases are important digestive enzyme synthesize in higher rate during silkworm larval stage (Kumari *et al*., 1997) highly responsible for dietary protein metabolism (Abudabos, 2012) in the digestive system of the silkworms. Many investigated the proteins (Trivedy *et al*., 2008) and proteolytic enzymes of the alimentary canal in insects including *Bombyx mori* from the viewpoint of nutrition and enzymology (Eguchi *et al*., 1982; Eguchi and Arai, 1983). Astaurov (1935) has opined that studies on artificial mutations in the silkworm through physical and chemical mutagens are of considerable interest for geneticists. Also there are number of chemicals for which silkworms show high level of sensitivity including some pesticides and disinfectants (Kanika *et al*., 2011). Since, information regarding DES effect on digestive enzymes is scanty; an attempt has been made in the present investigation to study the effect of chemical mutagen DES on the activity of digestive enzymes (amylase and protease) in haemolymph and midgut of silkworm, *Bombyx mori*.

MATERIAL AND METHODS

The healthy silkworm *Bombyx mori* larvae soon after the third moult considered for the experiment. Different concentrations (doses) of DES (Diethyl Sulphate) viz., 2, 4, 6, 8, 10 and 12 mM were prepared in distilled water and orally administered through mulberry leaves. For every 20 g of leaves 20 mL of appropriately diluted DES were used. Similarly, the injections for 5th day of Vth instar larvae of bivoltine (variety: NB_4D_2) using different doses as mentioned earlier. The dilutions were prepared in 0.75% sodium chloride solution and injected at the lateral side of the intersegmental region between the 7th and 8th abdominal segments using a micro syringe. Each larva was injected with 0.04 mL of solution. These treated larvae were maintained and observed up to spinning to find out the LD_{50} value (Konate *et al*., 2011) in the larval life following the method of Bhoopathy and Muthukrishnan (1985). Further, after assessing the LD_{50} value, two different doses of DES namely 8 and 10 mM were selected. Three replications were maintained for each treatment comprising of 100 larvae. These injected larvae were reared separately and allowed for spinning cocoon. The cocoons obtained from these treated, control (fed the leaves sprayed with distilled water and injected larvae with 0.75% Sodium Chloride) and untreated sets were harvested and preserved separately. The studies on digestive enzymes in midgut and haemolymph were made in the M_1 (F_1) generation. In M_1 generation, the 4th and 5th instar larvae for the assay of digestive enzymes. The midgut tissue of 4th and 5th instars larvae and haemolymph from 5th instar larvae were used for enzyme extraction following the method of Kumari *et al*. (1997). The quantitative analysis of amylase activity was done following the modified method by Ishaaya and Swirski (1976). Quantitative analysis of protease activity was done in haemolymph and midgut tissue according to Eguchi and Iwamoto (1982) and the enzyme assay was studied by the method used by Beena *et al*. (2012).

Statistical analysis: One-way analysis of variance was used to test the significance of differences between the mean values of independent observations of amylase activity and activity of Protease

in the midgut and haemolymph of silkworm larvae. Comparisons were performed with WINSTAT statistical package to find significance differences between the different treatments.

RESULTS

The effect of chemical mutagen diethyl sulfate (DES) on the silkworm physiology corresponding to the important digestive enzymes Amylase and Protease in the midgut and hemolymph were recorded as follows.

Amylase activity: the amylase activity in the midgut of silkworm larvae during 4th instar recorded an increased activity from first and attained peak on 2nd day in all the sets. The treatment with 10 mM injection showed higher amylase activity than other sets including untreated batch (Fig. 1a). In 5th instar, a higher activity was noticed on 3rd day in all treated, control and untreated sets and the maximum activity was on 6th day. The enzyme activity was found decreasing with the increase in the rate of dosage of the mutagen but the untreated set recorded maximum activity (Fig. 1b). The amylase activity of the haemolymph showed a similar trend as in midgut. The set treated with 8 mM DES injection also recorded a higher value among all the sets including untreated set (Fig. 1c).

Protease activity: The protease activity followed a similar trend like amylase activity during 4th and 5th instar (Fig. 2a-b). The treatment 8 mM injection showed a higher activity during 4th instar in midgut tissue of both the races. On the other hand during 5th instar in NB_4D_2, the untreated batch recorded higher protease activity than the treated and control batches on 3rd day. In haemolymph, a similar activity was found in all the sets (Fig. 2c). The higher activity was found in the set treated with 8 mM injection compared to control and untreated set.

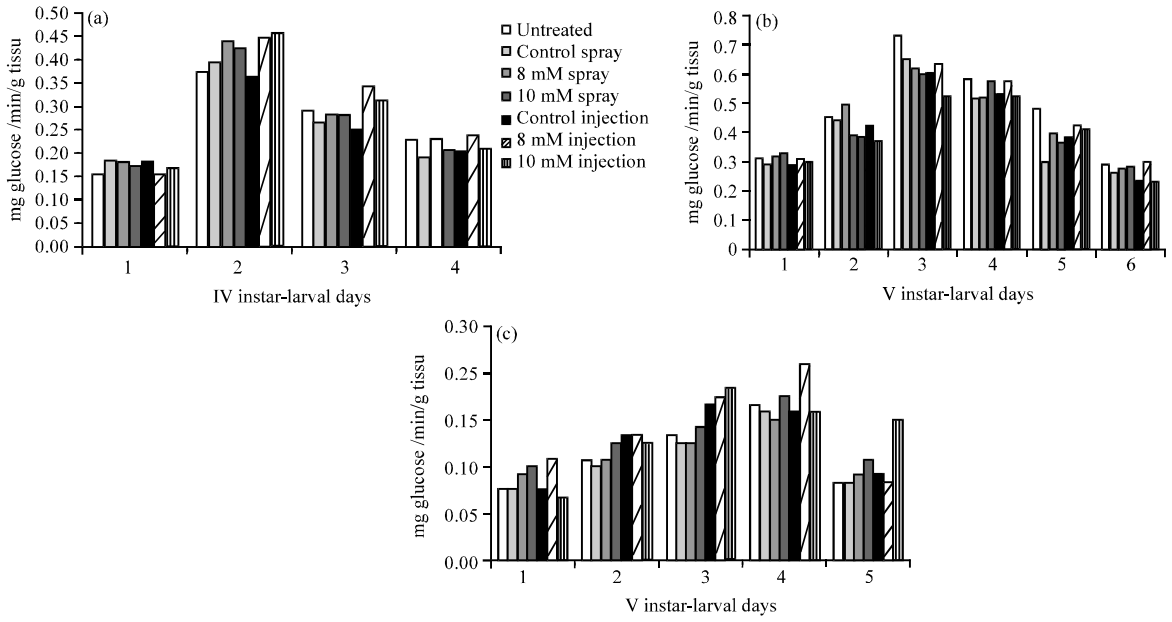

Fig. 1(a-c): (a, b) Amylase activity in midgut and (c) Amylase activity in haemolymph

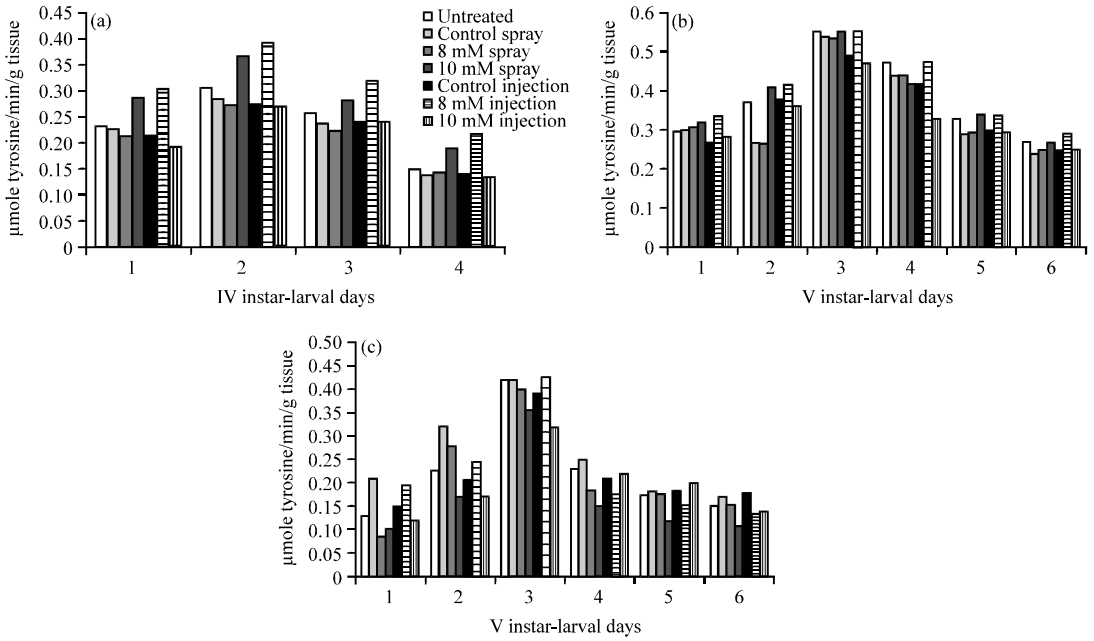

Fig. 2(a-c): (a-b) Protease activity in midgut and (c) Protease activity in haemolymph

DISCUSSION

In insects the complex food molecules are utilized after they have been processed into simpler molecules through the action of digestive enzymes in the gut of the larva. Thus, the enzyme system in the silkworm plays a vital role in determining the performance of the larvae in terms of effective transformation of organic food molecules of the leaf into useful biomolecules. The consumption of mulberry leaf during final instar accounts for more than 80% of the total consumption during its larval life. Food consumed in this stage is effectively utilized for the production of silk proteins as well as to support its metabolism (Lokesh *et al*., 2006). Thus the energy acquired by the larvae as a consequence of feeding is utilized in the subsequent non-feeding stages. In the present study, the impact of mutagenic agent (DES) on the digestive enzymes in both midgut and haemolymph revealed that the enzyme activity was maximum in the middle part of both 4th and 5th instars. This is mainly due to the fact that, the leaf consumed by the larvae was maximum during that period and the presence of food in the gut may act as a stimulus for the secretion of enzymes (Sarangi, 1985; Kumar *et al*., 2011). Ishaaya *et al*. (1971) showed that in the larvae of *Spodoptera littoralis*, the protein factors act as stimulants for digestive enzymes probably through a hormonal mechanism. Higher quantity of food accumulated in the digestive tract, leads to more secretion of digestive enzymes in the midgut (Waldbauer, 1968).

Being proteins, enzymes are known to be susceptible to chemical and radiation damage. The activity of enzyme may increase or decrease, depending upon the type of enzyme, exposure to mutagens and length of time elapsed between treatment and assay (Kumari *et al*., 1997). The highest activity of amylase in the midgut as well as in the haemolymph for the treatment with 8 mM injection and lower activity in 10 mM injection set implies that the lower concentration of the mutagenic agent could be attributed to the increased rate of metabolism and higher secretion of enzyme could enhance the higher activity. On the contrary, the lower enzyme activity could be

attributed to higher concentration of the mutagenic agent when induced directly, alters the genetic structure to a high degree resulting in deleterious effect on the enzyme activity. The maximum activity of the amylase in the midgut when compared with haemolymph could be attributed to the fact that the midgut tissue is the chief site for the secretion of carbohydrases. On the other hand, the enzyme activity is justified in the haemolymph because it is only the carrier of all the necessary substances including enzymes.

In contrast to the amylase activity, protease in both midgut and haemolymph followed a similar trend in the treated set. This could also be due to higher consumption of food and as well may be due to the impact of chemical mutagen.

Sarangi (1986) showed a higher activity of protease in bivoltine over the multivoltine race, wherein the activity was as high as 2.5 times. On the contrary, the amylase activity was observed to be 2 times higher in multivoltines when compared to bivoltine (Venugopal *et al*., 1987). In the present study, similar results were recorded for these enzymes. The difference in their activities was found to be significantly lower. This could be attributed to the effect of chemical mutagen in NB_4D_2 (Chatterjee and Datta, 1992).

CONCLUSION

From the present work it can be concluded that there is possibilities of acquire positive mutagenic response with lower doses of chemical mutagen DES and will have an impact in improving the physiological performances like enzyme activity in silkworms. The higher doses of DES apparently cause damage to the silkworm life. Higher enzyme activity obtained from 8 mM dose of DES found to be better and indicate that DES is potential mutagen in inducing beneficial effect with respect to the productivity in silkworms. At the same time judicious use of chemicals for the silkworm improvement is imperative.

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